



Technical Bulletin No. 4

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## Biotinylated IGFs from GroPep

### Binding to IGF Binding Proteins and the Type 1 IGF Receptor

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Biotinylated reagents are rapidly replacing traditional radioactive techniques for the sensitive, rapid and safe detection of an increasing number of biological molecules.

To support this trend GroPep has prepared three high purity biotinylated human IGFs.

Using NHS-biotin reagents which react with primary amines in the IGF molecule, GroPep has produced labelled molecules with excellent binding to avidin-linked detection reagents.

#### Biotinyl IGF-I:

- ◆ The major species is di-biotinyl IGF-I. Also contains mono- and tri-biotinyl species.
- ◆ Prepared with a linker arm chosen to minimize steric hindrance during subsequent avidin binding.

#### Mono-biotinyl IGF-II:

- ◆ Biotinyl group linked to the N-terminal alanine.

#### Di-biotinyl IGF-II:

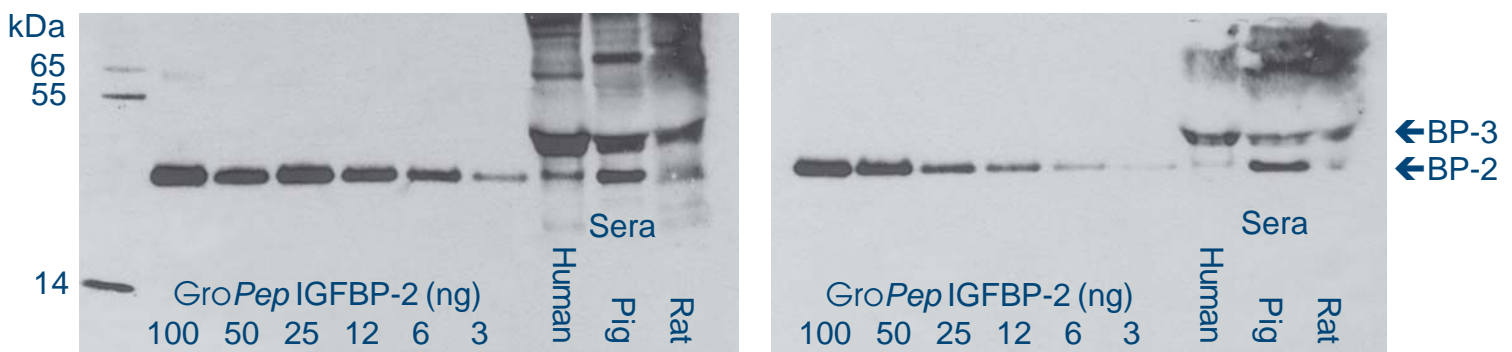
- ◆ Biotinyl groups attached to both the N-terminal alanine and lysine 65.
- ◆ Similar binding characteristics as mono-biotinyl IGF-II. (*Confirmed by Western Ligand Blot*).

Biotinyl IGFs are suitable for many applications. Two examples of their use are shown below. Other applications are envisaged, including as histochemical reagents.

## 1. Visualization of IGFBPs by Western Ligand Blot:

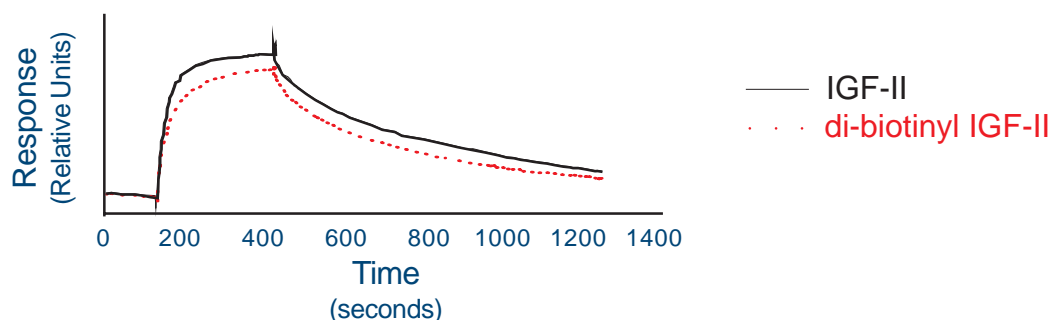
with biotinyl IGF-I...

...and mono-biotinyl IGF-II



Gel: 10-20% Tris-tricine SDS-PAGE, transferred to nitrocellulose  
Load: Serial dilution of GroPep bovine IGFBP-2  
2.5  $\mu$ l each of human, pig and rat serum  
Probed with: 0.2  $\mu$ g/ml GroPep biotinyl IGF-I or mono-biotinyl IGF-II  
Visualization: Streptavidin-horseradish peroxidase and chemiluminescent reagents  
Exposure time: 8 minutes

## 2. Type 1 IGF Receptor Binding:



Soluble Type 1 IGF Receptor (Surinya *et al.*, 1998) was immobilized on a CM5 chip and a 200 nM solution of either GroPep human IGF-II or di-biotinyl IGF-II passed over the receptor. This BIAcore analysis shows that both the native molecule and the biotinyl IGF-II exhibit similar association and dissociation rates, demonstrating equivalent binding to the IGF receptor.

### Useful References:

Surinya, K. H., *et al.* (1998) Production and characterisation of a soluble high affinity IGF-I receptor. Proc. Aust. Soc. Biochem. Mol. Biol., **30**, Pos. 132.

Grulich-Henn, J., *et al.* (1998) Ligand blot analysis of insulin-like growth factor-binding proteins using biotinylated insulin-like growth factor-I. Hormone Res., **49**, 1-7.

Op De Beeck, L., *et al.* (1997) Detection of serum insulin-like growth factor binding proteins on western ligand blots by biotinylated IGF and enhanced chemiluminescence. J. Endocrinol., **154**, R1-R5.

Fowlkes, J. L. and Serra, D. (1996) A rapid, non-radioactive method for the detection of insulin-like growth factor binding proteins by Western ligand blotting. Endocrinol., **137**, 5751-5754.

For specifications on any of these reagents, click on the product title below.

### Biotinyl human IGF-I

Code: AQU010	10 µg	US\$180
Code: AQU050	50 µg	US\$305
Code: AQU100	100 µg	US\$485

### Mono-biotinyl human IGF-II (Receptor Grade)

Code: AMU010	10 µg	US\$180
Code: AMU050	50 µg	US\$305

### Di-biotinyl human IGF-II (Receptor Grade)

Code: ANU050	50 µg	US\$305
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### General Properties:

- ◆ GroPep's biotinyl IGF products contain > 95% biotinylated IGFs by mass spectrometry.
- ◆ Biotinyl IGFs are supplied lyophilized and are stable for at least 2 years at 2 - 4°C.
- ◆ For other biotinylated reagents, please inquire.

**GroPep's biotinylated reagents are available now.**

**Order by FAX, E-mail or through our Website.**



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